EXHIBIT C3

2dET Am Jm eC28

=> inhib titration

Cells killed 7pm
harvest 12:30

		2x OD490	γ/r	· 1γ	2.5γ
2N		.292	2.1	0.48	1.2
2E		.353	2.8	0.36	0.9
2E		.209	1.1	0.93	2.2
2N	1mM IETD	.235	1.4	0.7	1.75
2E	"	.303	2.2	0.45	1.13
2N	1mM DEVD	.254	1.7	0.61	1.5
2E	"	.281	2.0	0.51	1.27
2N	5mM DEVD	.280	2.0	0.51	1.28
2E	"	.280	2.0	0.51	1.28
2N	10mM DEVD	.300	2.2	0.46	1.15
2E	"	.262	1.7	0.57	1.43
2N	1mM VEID	.336	2.5	0.40	1.00
2E	"	.255	1.7	0.60	1.50
2N	5mM VEID	.283	2.0	0.50	1.26
2E	"	.277	1.9	0.52	1.31
2N	10mM VEID	.217	1.2	0.82	2.01
2E	"	.256	1.7	0.60	1.49
2N	10mM YVAD	.288	2.0	0.49	1.22
2E	"	.257	1.7	0.59	1.48
2N	10mM LEHD	.303	2.2	0.45	1.13
2E	"	.288	2.0	0.49	1.22

Cells trypsinized
Buffer A remade => filtered,
pH to 8
Buffer B remade => filtered

HPA: NE/DC (1.0/25)
Dilute

5mM DTT, Prot, PMSF,
0.2mM vanadate

21 June 19

– Forgot to vortex after buffer A

*Sample 2N ⑧ got ice in 1.7

BEST AVAILABLE COPY

(3) 1 (3) 5 (3) 10

(1) 2N 12 (2E) 9 22 | 2N 15 2E 12.7 | 2N 12.9 2E 12.8 | 2N 9.5 2E 14.3

(6) 1 (6) 5 (6) 10

(2) 2N 12 (2E) 9 22 | 2N 10.1 2E 15 | 2N 12.6 2E 13.1 | 2N 20.4 2E 9 14.9

(8) (1) (9)

(3) 2N 12 (2E) 9 22 | 2N 12.5 2E 17.3 | 2N 12.2 2E 14.8 | 2N 11.3 2E 12.2

(4) 1γ → βCAT | 2.5γ - pro | 10γ - λCAT

(M) 2N 4.8 (2E) 8.8 3.6 | (M) 2N 12 (2E) 9 22 | 2N 4.8+4.8 2E 8.8+8.8

Note: loaded 1γ instead of 2.5γ of 2E for ECAD gels 4 prog
loaded 0.4γ instead of 1γ 2E for βCAT gel 1

Repeat

(3) 1mM (3) 5mM (3) 10mM

(1) 2N 4.8 2E 8.8 | 2N 6.1 2E 5.1 | 2N 5.1 2E 5.1 | 2N 4.6 2E 5.7

2N =
2E =

(6) 1mM (6) 5mM (6) 10mM

(2) 2N 4.8 2E 8.8 | 2N 4.0 2E 6.0 | 2N 5.0 2E 5.2 | 2N 8.2 2E 6.0

(8) (1) (9)

(3) 2N 4.8 2E 8.8 | 2N 7 2E 4.5 | 2N 4.9 2E 5.9 | 2N 4.5 2E 4.9

1γ βCAT | 2.5γ - pro | ~~10γ λCAT~~

(4) (M) 2N 4.8 2E 8.8 | (M) 2N 12 2E 22 | (M) ~~2N 2E~~ ~~(M) 2N 2E~~

Results:

~~Se~~ Cell (4) / Set 1

β Cat: see small amt. of cleaved product not seen in unstim. sample

p120 cat: definitely see shift, esp. in lower Mwt. isoforms

E CAD: no shift but see ~ 2X decrease

Cells (1), (2), (3), Set 2

– No signal or very faint ⇒ Redev. c̄ Pierce Reagent

Cell (1) – DEVD (3)

- control screwed up ; ~~[illegible]~~
- 1nM (3), slight ↓ E CAD
- 5nM (3), ↓ in ECAD @ baseline, 3-4X ↓ in ECAD by ET-1
- 10nM (3), ECAD level down to ~~[illegible]~~ barely detectable

Cell (2) – VETD (8)

- control screwed up
- (8) 1nM, 5nM: ECAD levels similar between stimulated and unstimulated samples (ET-1)
- 10nM ~~[illegible]~~ marked downreg of ~~ET~~ ECAD in ET-1 stimulated cells

Cell (3)

- control screwed up
- (3) 1nM: significant inhibit of ↓ ECAD
- (3) & 9nM: Marked downreg of ECAD by ET-1

BEST AVAILABLE COPY

Concl:
- too little protein + to short Ab inc. time
- control screwed-up 2° to quantitation error
  ↳ - requantitate samps
  - load 25γ extract
  - 1°/45' (1°/2°) Ab incubation

- YVAD, LEHD @ 10nM ∅ effect on ↓ECAD by ETY
  ①
  ∴ Caspase 1, 4, 5, 9 not likely to be involved
  ↳ do one more trial of ①, ⑨ @ 1nM

- DEVD, IETD, and VEID interfere c̄ ↓ECAD by ETY.
  - have already ruled out Caspase 3, 7
  - have ruled in Caspase 8
  - need to ✓ Caspase 6, 10.